



Radegen Biotechnology™

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2023.11.24 Project Skunkworks: Radegen DNA Assembly™ T5-SfiI™

The first core competency of Radegen Biotechnology is cloning reagents. Radegen DNA Assembly™ T5-SfiI™ is a purified protein reagent composed of T5 exonuclease, a high fidelity DNA ligase like Taq or Tth ligase, a proofreading polymerase like Psp polymerase and SfiI endonuclease. This system is a next-generation Golden Gate system and is characterized as so because it recombines multiple donor plasmids by first existing a dsDNA donor fragment from a plasmid and clones the fragment into a SfiI domesticated plasmid. The proofreading polymerase in the enzyme cocktail removes terminal end mismatch nucleotides and the T5 exonuclease removes any 5' mismatches while producing a 3' overhang. In a properly designed donor DNA with homology arms targeting the dsDNA fragment to a destination plasmid is said to have long homology sticky end overhangs. The enzymes in this system are chosen for an iso-thermal one pot DNA assembly reaction at 50 degrees celsius and the final product from this reaction consists of a seamless DNA construct. The revolutionary aspect of this system is the 8 bp rare cutter endonuclease, SfiI which minimizes the need to domesticate a destination plasmid and donor DNA from the common 1 in 4⁶ or the less frequent 1 in 4⁷ binding frequencies to 1 in 4⁸ frequency of SfiI. Creative Commons licensing protects the use of T5 in combination with an endonuclease in any iteration. The T5-SfiI combination of enzymes is a purified protein version of T5-NotI and T5-SapI that is amenable to incubation at 50 degrees Celsius, facilitating an efficient one pot reaction with the other components of the system.

>T5 Exonuclease
MSKSGWKFIEEEAEMASRRNLMIVDGTNLGFRRFKHNNSKPFASSYVSTIQSLAKSYSARTTIVLGDKGKSVFRLPEYKGRNRDEKYAQRTEEAKALDEQFFEYLKDCAFELCKTTFPTFTIRGVVEADMAAYIVKLIGHLYDHVWLISTGDWDTLLTDKVSRFSFTTREYHLRDMYHEHHNVDDVEQFISLKAIMGDLGNIRGVGEGIGAKRGYNIREFGVNLIDQLPLPGKQKYIQNLNASEELLFRNLILVLPLTCYVDAIAAVGQDVLDKFTKDILEIAEQ*

>SfiI
MHQDYRELSDLELESVEKQTLLRTIVQALQQYSKEAKSIFETTAADSSGEVIVLAEDITQYALEVAETYPINRRFAFGIDYKRVRLPSPHGLLPQVLLVDAKASTEKNRDTLQRSQLPMDAEFRNTSSGEVVTMEAGVIPHMLQ SANDGVLPAVTTSIFVHFYYRELKDVEGRYRELKSTIVLSPHARLKQRYNPDPDTSSFFGAGKHSPARGEVARIRVFDRLKEACPWRWLQELHYSADSEYTQPRWRDLDAGHEVTKEFLFLER*

>SfiI.M1
MRKQSSGQPLAGDADETRSRSLATHAGPDFALYVGDSLDCLAKPDDESINTVVTSPPYWAVRDYHEQDOLGLQLEDEVDDYVERLVKIVFREVYRVLATDGSASWLNIDSYFNQITVGGKPPRTGWKRKNQLSLVPFRVALALQDDGWIRNVAVWHKP NAMPASVRDRLRTVWEFPFLTKSERVYFNLDIEIRVPHQTSDAIERRAESGTVTGKAQGKKELRKWLNSPRHATIEGIKEVERRNPAAPAVELASYLRTALKERKSIAWVAEQLDPFERTRHYFRTEIGSRLPPEVWEQLKDLLELDATY DEAMTVEVGDNVFRNHPNGKPNQGDLSSIPTAPSAGHFAVMPRKLAHFALKATLPMNGSCLDPFMGSSTGRRVRELGGRFVGVDVNEHYMTDYLVESGVISPETELW*

>taq ligase
MTLEAARRRVNELRDLIRHYNYLYYVLDPEISDAEYDRLLRELKELEERFPELQSPDSPTEQVGARPLEATFRPVHRPTRMYSLDNAFLDEVRAFEERIERALGRKGPFLYTVHKVDGLSVNLYYEEGLVFGATRGDGETGEVEVTQNLLTIR TIPRRLTGVPDRLEVRGEVYMPIEAFRLRNEELEAGERIFKNPRAAAGSLRQKDPRTAARRGLRATFYALGLGLEETGLKSQHDLLLWRERGFPVHGTFRALGAEGVEEVYQAWLKERRKLPEADGVVVKLDDALWRELGYTARAPRFA AYKFPAAEKKETRLLSVAFQVGRTRGTPVGLPEVIEGSVRSVTLHNESYFIEELDIRGDWLVHKAGGVPEVRLVKERRTGEERPIRWPETCPEGHRLKEGKVKHCPNPLCPAKRFEAIRHYASRKAMDIQGLGEKLIKLLKGLVRD VADYLRLKEDDLVNRMEGEKAENLLRQIEESKGRGLERLRLYALGLPGVGEVLRNLARLRFHMDRRLLEAGLEDLLEVEGVGELTARAILETLKDOPFDRLVRLKEAGVEMEAKEREGEALKGLTFITGELSRRPREEVKALLRLGAKVTDVS RKTGFLVVGGENPGSKLEKARALGVPTLSEEELYRLIEERTGKDPRALTA*

>tth ligase
MTLEARKRVNELRDLIRHYNYRYYVLDPEISDAEYDRLLRELKELEERFPELQSPDSPTEQVGARPLEATFRPVHRPTRMYSLDNAFLDEVRAFEERIERALGRKGPFAYTVHKVDGLSVNLYYEEGLVFGATRGDGETGEVEVTQNLLTIP TIPRRLKGVPERLEVRGEVYMPIEAFRLRNEELEAGERIFKNPRAAAGSLRQKDPRTAARRGLRATFYALGLGLEETGLKSQHDLLLWRERGFPVHGTFRALGAEGVEEVYQAWLKERRKLPEADGVVVKLDDALWRELGYTARAPRFA AIAFKFPAAEKKETRLLDVVFQVGRTRGTPVGLPEVIEGSVRSVTLHNESYFIEELDIRGDWLVHKAGGVPEVRLVKERRTGEERPIRWPETCPEGHRLKEGKVKHCPNPLCPAKRFEAIRHYASRKAMDIQGLGEKLIKLLKGLVRD VDKDADLYRLKEDDLVGLERMEGEKAENLLRQIEESKGRGLERLRLYALGLPGVGEVLRNLARLRFHMDRRLLEASLEELLEVEGVGELTARAILETLKDOPFDRLVRLKEAGVEMEAKEREGEALKGLTFITGELSRRPREEVKALLRLGAKVTDVS RSVRKTSYLVGENPGSKLEKARALGVPTLSEEELYRLIEERTGKKAELV*

>psp polymerase
MIIDADYITEDGKPIRIKIFKKEKEFVKVEYDRTFRPYIYALLKDDSAIDEVKKITAERHGKIVRITEVKVQKKFLGRPIEWKLYLEHPQDVAIREKIREHPAVVDIFEYDIPFAKRYLIDKGLTPMEGNEELTFLAVDIETLYHEGEFGKGP IIMISYADEEGAKVITWKSIDLPVVEVSSEREMIKRLVKVIREKDPPDVIITYNGONDFDPYLLKRAEKLGIKLPLGRDNSEPKMQRMDSLAVEIKGRIHFDLFPAIRRTINLPTTYLETVEVIFGSKSEKVAHEIAEAWETGKGLERVAKYS MEDAKVSELGKEFFPMEAQLARLVGHPWDVRSSTGNLVEWFLLTAKERNEELAPNKPDEREYERRLRESYEGGYVNEPEKGLWEGIVSLDFRSLSYPSIIITHNVSPDTLNRENCKEYDVAPQVGHFRFCKDFFGFIPLSLLGNLEERQKIKRM KESKDPVEKKLLDYRQRAIKLANSYYGGYAKARWYCKECAESVTANGROYIDLVRRELESRGFKVLYIDTDGLYATIPGAKHEEIKEKALKFVEINSKLPGLELEYEGFYARGFVTKKYALIDEEGKIVTRGLEIVRRDWSEIAKETQA KVLEAILKHNGVDEAVKIVKEVTEKLSKYEIPPEKLYIEQITRPLSEYKAIGPHVAVKRALAKGVVKPGMVIGYIVLKGDPGISKRAIAIEFDPKKKHDAEYIENQVLPRAVERILRAGYRKEDLRYQTKVQVGLGAWLK*